肥胖人群与正常人群尿液蛋白质组的比较

王海彤 [1]，魏利龙 [2]，周允 [2]，曹永彤 [2]*，高友鹤 [1]*
[1]（北京师范大学 生命科学学院 基因工程药物及生物技术北京市重点实验室
北京 100875），[2]（中日友好医院 检验科 北京 100029）

摘要
目的：对肥胖人群与正常人群尿液蛋白质组的比较。
方法：收集肥胖人群和正常人群的尿液样品，通过高效液相色谱串联质谱联用（LC-MS/MS）的非标记定量蛋白质组学技术进行鉴定。筛选肥胖人群与正常人群尿液蛋白质组的差异蛋白进行蛋白质功能和生物学通路分析；将肥胖个人与正常人群尿液蛋白质组进行比较，统计共有差异蛋白进行蛋白质功能和生物学通路分析；在肥胖个人尿液蛋白质组中检索已被报道的肥胖生物标志物。
结果：肥胖人群相对于正常人群尿液蛋白质组可鉴定到 38 个差异蛋白，其中有些蛋白已经被报道与代谢、肥胖相关，差异蛋白富集到的生物学过程也与代谢等过程相关；肥胖个人与正常人群尿液蛋白质组比较富集到 8 个共有差异蛋白，其中有蛋白已经被报道与代谢、肥胖相关，差异蛋白富集到的生物学过程也与代谢等过程相关；在肥胖个人相对于正常人群尿液蛋白质组的差异蛋白中，能匹配到已被报道的肥胖生物标志物。
结论：尿液蛋白质组能进行正常人与肥胖者的区分，尿液蛋白质组差异蛋白中具有已知和肥胖、代谢相关的关键蛋白，且差异蛋白能富集到营养、代谢等相关生物学过程。尿液蛋白质组具有探究肥胖发生机制、提供个性化治疗的潜力。
关键词： 尿液蛋白质组 肥胖 体质指数

Comparison of urine proteome between obese people and normal people

Haitong Wang[1] Lilong Wei[2] Yun Zhou[2] Yongtong Cao[2] Youhe Gao[1]*
[1](Gene Engineering Drug and Biotechnology Beijing Key Laboratory, College of Life Sciences，Beijing Normal University, Beijing 100871, China) [2](Clinical Laboratory, China-Japan Friendship Hospital, Beijing 100029)

Abstract
Objective: Comparison of urine proteome between obese people and normal people.
Methods: Urine samples from obese and normal people were collected and identified by non-label quantitative proteomics using high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). The difference proteins of urine proteome between obese and normal people were screened for protein function and biological pathway analysis. The urine proteome of obese individuals was compared with that of normal people, and the common differential proteins were counted to analyze the protein function and biological pathways. Reported biomarkers of obesity were searched in the urine proteome of obese individuals.
Results: 38 different proteins can be identified in the urine proteome of obese people compared with normal people, some of which have been reported to be related to metabolism and obesity, and the biological processes of differential proteins are also related to metabolism and other processes. 8 common differential proteins in the urine proteome of obese individuals and normal people, among which some proteins have been reported to be related to metabolism and obesity, and the biological processes of differential proteins are also related to metabolism and other processes. Among the differential proteins in the urine proteome of obese individuals compared with the normal people, the reported obesity biomarkers can be matched.

基金项目：国家自然科学基金(82272407)；北京市临床重点专科项目(2020 年)
作者简介：1.王海彤.（一作），女，硕士研究生，主要研究方向：尿液生物标志物；2.魏利龙.（一作），男，主治医师，主要研究方向：体液疾病标记物；3.周允.（二作），男，副研究员，主要研究方向：疾病标记物。
通信联系人：1.高友鹤（第一通讯），男，教授，博士生导师，主要研究方向：尿液蛋白质组学与尿液生物标志物.E-mail: gaoyouhe@bnu.edu.cn；2. 曹永彤（第二通讯），男，主任医师，主要研究方向：医学检验技术与疾病标记物.E-mail: caoyongtong100@sina.com.

Conclusions: The urine proteome can distinguish the obese people, and the differential proteins in the urine proteome have key proteins that are known to be related to obesity and metabolism, and the biological processes of differential proteins also related biological processes such as nutrition and metabolism. Urine proteome has the potential to explore the pathogenesis of obesity and provide personalized treatment.
Keywords: Urine proteome; obesity; Body mass index

1 引言

全球流行的肥胖是的当今重大公共卫生问题，肥胖会增加许多慢性疾病的风险，如 2 型糖尿病、冠心病和某些癌症。肥胖的致病因素复杂，是遗传、营养和代谢因素相互作用的结果。虽然肥胖分类和表征的术语现在并没有明确的共识，但仍大致可分为以下 4 种表型：(1)正常体重肥胖(normal weight obese , NWO)；(2)代谢正常肥胖体重(metabolically obese normal weight, MONW)；(3)代谢健康型肥胖(metabolically healthy obese, MHO)；(4)代谢不健康肥胖(metabolically unhealthy obese, MUO)；(5)肌少性肥胖(sarcopenic obese , SO)[1 2]。

目前还没有显著的生物标志物用于肥胖和各个肥胖亚型的区分，用于肥胖分类的是身体质量指数(Body Mass Index, BMI)，计算方法为体重（kg）除以身高（m）的平方,根据中国的 BMI 分级，偏瘦定义为 BMI < 18.5 kg/m^2，正常为 18.5 kg/m^2 ≤ BMI < 24 kg/m^2，超重为 24 kg/m^2 ≤ BMI < 28 kg/m^2，肥胖为 BMI ≥ 28 kg/m^2，是一种不完善的衡量身体脂肪异常的指标[1 3 4]。

更多的精细技术，如磁共振成像，用于评估身体脂肪分布来更好地诊断肥胖亚型，但这些检测在常规临床中并不容易，且临界值尚未建立[1]。而用更简单的手段，快速鉴定特异性生物标志物表征肥胖成因，并在个性化治疗中提供靶点，还仍需进一步研究。尿液是血液经过肾脏过滤产生，排除体外的用以代谢废物，由于其不属于机体内环境，不受稳态调节机制的控制，因此能够保留机体极微小的生理变化[5]。有研究表明，尿液蛋白质组可以监测到疾病的生物标志物，如：糖尿病[6]、阿尔兹海默症[7]、抑郁症[8]、自闭症[9]；尿液蛋白质组的生物标志物还可以对疾病进行分类，如：预测慢性肾病[10]、区分卵巢癌良恶性肿瘤[11]等。但是尿液蛋白质组学还没有在寻找生物标志物进行肥胖成因和个性化治疗方面的研究，因此本研究通过对肥胖人群和正常人群进行尿液蛋白质组学分析，以探索尿液是否能反应肥胖成因、提供潜在药物靶点、辅助进行个性化治疗。

2.1 实验方法

2.1.1 样本收集

本次实验共收集了来自北京中日友好医院的 19 位被试者的尿液样本。根据 BMI 中国参考标准， 18.5 < BMI < 23.9 为正常；BMI > 28 为肥胖，本次收集样本中肥胖组样本 10 例，平均 BMI = 35.79 kg/m^2；正常组样本 9 例，平均 BMI =22.76 kg/m^2。本次实验基于检验科的废弃样本再利用，其过程未涉及病人的任何身份信息，伦理审查编号：2023-KY-126。受试者 BMI 如表 1 所示：

表 1 肥胖组与正常组尿液蛋白质组差异蛋白

	编号	年龄	BMI（单位：kg/m^2）
正常组	1	32 岁	21
正常组	2	31 岁	21
正常组	3	34 岁	22
正常组	4	33 岁	23
正常组	5	33 岁	23.
正常组	6	34 岁	23
正常组	7	33 岁	24
正常组	8	35 岁	24
正常组	9	27 岁	24

肥胖组	11	33 岁	30
肥胖组	12	33 岁	30
肥胖组	13	30 岁	30
肥胖组	14	29 岁	31
肥胖组	15	31 岁	32
肥胖组	16	33 岁	34
肥胖组	17	28 岁	37
肥胖组	18	29 岁	41
肥胖组	19	19 岁	44
肥胖组	20	19 岁	49

2.1.2 尿液样本处理

尿蛋白提取：-80℃冰箱中取出尿液样本， 4°C 的条件下解冻。4℃，12000×g 离心 30 min，取 6 mL 上清液，加入 20 mM 二硫苏糖醇溶液（Dithiothreitol, DTT, Sigma），涡旋混匀，水浴 37°C 加热 60 min，冷却至室温。加入 50 mM 碘乙酰胺（Iodoacetamide, IAA, Sigma），涡旋混匀，室温避光反应 40 min。反应结束后加入三倍体积的预冷无水乙醇，上下颠倒轻柔混匀，-20°C 沉淀蛋白 24 h。沉淀的混合液 4°C，12000×g 离心 30 min，弃上清，等待乙醇挥发干燥。将蛋白沉淀重悬于裂解液中(含 8 mol/L 尿素，2 mol/L 硫脲，25 mmol/L 二硫苏糖醇，50 mmol/L Tris)。 4°C， 12000×g 离心 30 min，取上清于新的 1.5 mL 离心管内，获得尿液蛋白质。用 Bradford 法测定蛋白质浓度。

尿蛋白酶切：取 100 μg 尿液蛋白质样品于 1.5 mL 离心管中，加入 25 mmol/L NH_4HCO_3 溶液使总体积为 200 μL。取 10 kDa 超滤管(Pall, Port Washington, NY, USA) 向滤膜上加入 200 μL UA 溶液 (8 mol/L 尿素，0.1 mol/L Tris-HCl，pH 8.5)洗涤滤膜，18°C，14000×g 离心 5 min，弃去下层滤液，重复一次；向滤膜上加入碘乙酰胺处理完成后的尿液蛋白质样品，18℃，14000×g 离心 30 min，弃去下层滤液，尿液蛋白质留在滤膜上；向滤膜中加入 200 μL UA 溶液洗涤尿液蛋白质，18°C，14000×g 离心 30 min，重复两次；向滤膜中加入 25 mmol/L NH4HCO3 溶液洗涤尿液蛋白质，18°C，14000×g 离心 30 min，重复两次；按胰酶：蛋白为 1：50 的比例加入胰蛋白酶（Trypsin Gold, Promega, Fitchburg, WI, USA）进行酶切，37°C 水浴 16 h。酶切结束后 4℃，13000×g 离心 30 min 收集滤液，该滤液为多肽混合液。将多肽混合液通过 HLB 固相萃取柱(Waters，Milford，MA)进行除盐，使用真空干燥仪冻干，于-20°C 条件下保存。

2.1.3 LC-MS/MS 串联质谱分析

0.1%甲酸溶解多肽混合液冻干，使用 BCA 试剂盒对肽段进行定量，将肽段浓度稀释为 0.5 μg/μL。每个样品取 6 μL 混匀，使用高 pH 反相肽分离试剂盒(Thermo Fisher Scientific)进行分离。离心收集 10 份流出液（Fractions），使用真空干燥仪冻干后用 0.1%甲酸复溶。以对 10 份流出液和全部单个样品以样品 : iRT 体积比为 10 : 1 的例加入 iRT 试剂(Biognosys, Switzerland)，以校准提取的肽峰的保留时间。

10 份流出液使用 EASY-nLC1200 色谱系统(Thermo Fisher Scientific, USA)进行分离，分离的肽段经过 Orbitrap Fusion Lumos Tribrid 质谱仪(Thermo Fisher Scientific, USA)以 Data Dependent Acquisition(DDA)模式进行质谱分析并采集数据，生成 10 份 raw 文件，导入 Proteome Discoverer 软件采用 Swiss-iRT 和 Uniprot-Rat 数据库进行建库分析（version 2.0, Thermo Scientific）。根据建库结果设定单个样品 Data Independent Acquisition(DIA)模式的 39 个可变窗口建立 DIA 方法。单个样品取 1 μg 肽段，使用 EASY-nLC1200 色谱系统(Thermo Fisher Scientific, USA)进行分离，分离的肽段经过 Orbitrap Fusion Lumos Tribrid 质谱仪(Thermo Fisher Scientific, USA)以 DIA 模式进行质谱分析，采用新建立的 DIA 方法进行 DIA 采集数据，生成 raw 文件。

2.1.4 Label-free DIA 定量分析

将 DIA 模式下采集的单个样品 raw 文件导入 Spectronaut Pulsar(Biognosys AG, Switzerland)软件进行分析。由 MS2 中各片段离子的峰面积相加，计算肽段丰度。由各自的肽段丰度相加计算蛋白质丰度。

2.1.5 数据分析

每个样本进行 3 次技术重复，取平均值进行统计学分析。本实验进行肥胖组和正常组的成组比较对比，筛选差异蛋白。差异蛋白筛选条件为：组间变化倍数（FC, Fold change）≥ 2 或 ≤ 0.5，双尾非配对 t 检验分析的 P 值 ＜ 0.01；同时本实验进行一对多比较分析，将肥胖组单个样本与正常组 9 个样本比较，筛选差异蛋白，差异蛋白筛选条件为：组间变化倍数（FC, Fold change）≥ 1.5 或 ≤ 0.67，双尾非配对 t 检验分析的 P 值 ＜ 0.055；再统计肥胖组 10 个样本共有的共有差异蛋白。筛选到的差异蛋白通过 Uniprot 网站（https://www.uniprot.org/）分析，并在 Pubmed 数据库（https://pubmed.ncbi.nlm.nih.gov）中检索相关文献，对差异蛋白进行功能分析。

3 实验结果与分析

3.1 肥胖组与正常组尿液蛋白质组比较

3.1.1 差异蛋白

将肥胖组与正常组尿液蛋白质进行比较，筛选差异蛋白条件为：FC ≥ 2 或 ≤ 0.5，双尾非配对 t 检验 P < 0.01。结果表明，肥胖组与正常组相比，可以鉴定到 38 个差异蛋白，将差异蛋白按 FC 由小到大的顺序排列，通过 Uniprot 进行检索，结果如表 2 所示。

表 2 肥胖组与正常组尿液蛋白质组差异蛋白

Uniprot ID	Protein names	Fold change	Trend	P value
A0A1B0GVG4	Coiled-coil domain-containing protein 194	0.000	↓	0.006
Q9Y3C6	Peptidyl-prolyl cis-trans isomerase-like 1	0.002	↓	0.005
Q5T6V5	Queuosine salvage protein	0.010	↓	0.002
O95359	Transforming acidic coiled-coil-containing protein 2	0.106	↓	0.006
P48739	Phosphatidylinositol transfer protein beta isoform	0.162	↓	0.002
Q9NQX5	Neural proliferation differentiation and control protein 1	0.167	↓	0.003
Q6P2Q9	Pre-mRNA-processing-splicing factor 8	0.169	↓	0.003
O94933	SLIT and NTRK-like protein 3	0.180	↓	0.000
Q9BYX7	Putative beta-actin-like protein 3	0.222	↓	0.007
Q15797	Mothers against decapentaplegic homolog 1	0.223	↓	0.009
Q96S82	Ubiquitin-like protein 7	0.233	↓	0.000
Q99962	Endophilin-A1	0.233	↓	0.007
Q04323	UBX domain-containing protein 1	0.252	↓	0.009
Q9H251	Cadherin-23	0.252	↓	0.001
O76036	Natural cytotoxicity triggering receptor 1	0.254	↓	0.009
O96013	Serine/threonine-protein kinase PAK 4	0.257	↓	0.001
Q99798	Aconitate hydratase, mitochondrial	0.258	↓	0.009
P56199	Integrin alpha-1	0.268	↓	0.001
P08833	Insulin-like growth factor-binding protein 1	0.286	↓	0.002
P31150	Rab GDP dissociation inhibitor alpha	0.291	↓	0.000
Q53LP3	Ankyrin repeat domain-containing protein SOWAHC	0.299	↓	0.004
Q2M243	Coiled-coil domain-containing protein 27	0.317	↓	0.007
Q9Y2Q5	Ragulator complex protein LAMTOR2	0.337	↓	0.008

Q96EG1	Arylsulfatase G	0.355	↓	0.009
Q96TC7	Regulator of microtubule dynamics protein 3	0.362	↓	0.004
O43813	Glutathione S-transferase LANCL1	0.363	↓	0.007
Q99426	Tubulin-folding cofactor B	0.364	↓	0.001
Q9NQX7	Integral membrane protein 2C	0.379	↓	0.008
P20933	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase	0.391	↓	0.006
O75581	Low-density lipoprotein receptor-related protein 6	0.395	↓	0.003
Q6ZVM7	TOM1-like protein 2	0.402	↓	0.004
P43487	Ran-specific GTPase-activating protein	0.447	↓	0.002
Q9Y3Q0	N-acetylated-alpha-linked acidic dipeptidase 2	0.451	↓	0.005
P14209	CD99 antigen	2.006	↑	0.010
P55103	Inhibin beta C chain	2.010	↑	0.003
Q8WY21	VPS10 domain-containing receptor SorCS1	2.391	↑	0.010
P37235	Hippocalcin-like protein 1	2.551	↑	0.003
Q9P2J2	Protein turtle homolog A	2.761	↑	0.006

3.1.2 差异蛋白功能分析

将鉴定到的 38 个差异蛋白经过 PubMed 数据库进行文献检索。

其中变化最显著的若干差异蛋白，目前暂未有报道与肥胖、糖尿病等疾病相关。但其中差异蛋白中 Insulin-like growth factor-binding protein 1 (IGFBP-1) ，FC= 0.286，P = 0.002，即肥胖组相对于正常组下调 3 倍以上，有大量研究表明该蛋白与 BMI、腰臀比、空腹胰岛素水平之间存在负相关关系[12 13]。

IGFBP-1 水平主要通过胰岛素进行动态调节，当餐后胰岛素大量分泌时，会抑制 IGFBP-1 上游启动子，抑制其表达，IGFBP-1 水平迅速下降，由此增加了 Insulin-like growth factor 1 的生物活性增强其胰岛素样作用[14 15 16]。

已有研究在欧洲和巴基斯坦人[17]，亚洲印第安人[18]，健康年轻人[19]，65 岁以上成年人[20]，肥胖绝经期妇女[21]，1 型糖尿病患者[22]和青春期前儿童[12、23]等不同人群中证实，IGFBP - 1 浓度与胰岛素敏感性之间呈正相关。因此，IGFBP - 1 被认为是胰岛素敏感性的潜在标志物[24]。 通过对 615 例个体进行研究， 确定 IGFBP - 1 浓度及其与 IGF - 1 的相互作用是葡萄糖不耐受或糖尿病发展的重要决定因素[25]。通过对 355 名瑞典男性进行研究证明，空腹低 IGFBP - 1 浓度可以预测糖调节能力异常的发展，其中一些人患糖尿病的风险增加了 40 倍[26]。还有研究对 782 例个体进行了 17 年的随访，发现 IGFBP - 1 低表达预示着 2 型糖尿病的发生[27]。一项对 240 名女性 8 年以上的研究也表明，IGFBP - 1 与糖尿病风险增加有关[28]。

除此以外还有其他差异蛋白已经被报道与代谢或肥胖相关。Aconitate hydratase 参与三羧酸循环和碳水化合物代谢，具有催化柠檬酸盐通过顺乌头酸异构化为异柠檬酸盐的功能，能够通过介导细胞 ATP 的产生来控制脂肪生成[29]。抑制素和激活素分别抑制和激活垂体分泌促卵泡素。Inhibin beta 参与许多功能的调节，如：下丘脑和垂体激素分泌、性腺激素分泌、生殖细胞发育和成熟、红细胞分化、胰岛素分泌、神经细胞存活、胚胎轴向发育或骨骼生长，而 BMI 是其水平的重要独立预测因子[30]。

肥胖组相对于正常组的差异蛋白变化较为显著的蛋白，已经被报道与肥胖或代谢相关；而比这些差异蛋白变化更显著的蛋白，虽现在未有研究报道其与肥胖或代谢的关系，但仍值得对这些蛋白在肥胖和代谢方面的功能做进一步的研究，期以寻找肥胖的生物标志物或潜在药物靶点。

3.1.3 差异蛋白富集生物学过程分析

利用 DAVID 数据库对肥胖组与正常组的差异蛋白进行生物学通路的分析。共富集到 24 个 $P < 0.01$ 的生物学过程，结果如图 1 所示。

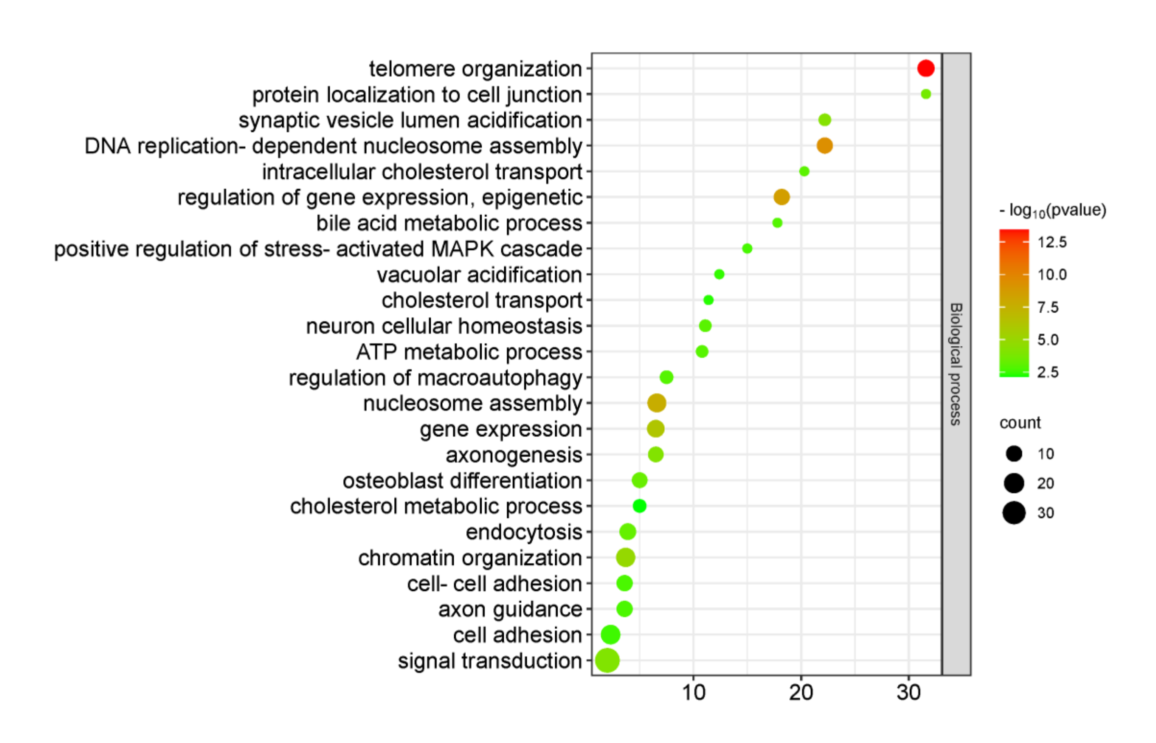


图 1 肥胖组与正常组尿液蛋白质组差异蛋白富集生物学过程

其中包括不少代谢相关的生物学过程，如：intracellular cholesterol transport、ATP metabolic process、bile acid metabolic process、cholesterol transport 和 cholesterol metabolic process。但是还有一些涉及基因表达和神经系统相关的生物学过程，其 P 值相对于这些生物学过程更小。其中 telomere organization 可能与肥胖相关，有研究表明，端粒长度是生物衰老的强有力标志，并且在肥胖的成年人中注意到端粒磨损增加[31]。其余生物学过程现在还未查到这些生物学过程与肥胖或 BMI 相关，这为我们理解肥胖的成因提供了新的研究方向和思路。

3.2 肥胖组单个个体与正常组尿液蛋白质组比较

3.2.1 肥胖组个体共有差异蛋白

将肥胖组单个个体与正常组尿液蛋白质组进行比较，筛选差异蛋白条件为：$FC \geq 1.5$ 或 ≤ 0.67，双尾非配对 t 检验 $P < 0.05$，统计肥胖组个体共有的差异蛋白。结果表明，鉴定到 8 个共有差异蛋白，如表 3 所示。值得注意的是，肥胖组单个个体的所有差异蛋白相对于正常组均下降，且除 Peptidyl-prolyl cis-trans isomerase-like 1 和 Queuosine 5'-phosphate N-glycosylase/hydrolase 外，其余 6 个差异蛋白在正常组中表达，而在肥胖组单个个体中均不表达。

表 3 肥胖组单个个体与正常组尿液蛋白质组的共有差异蛋白

Uniprot ID	Protein names	Trend
Q9Y5F0	Protocadherin beta-13	↓
Q8IY92	Structure-specific endonuclease subunit SLX4	↓
Q96AA8	Janus kinase and microtubule-interacting protein 2	↓
Q9Y3C6	Peptidyl-prolyl cis-trans isomerase-like 1	↓
A0A1B0GVG4	Coiled-coil domain-containing protein 194	↓
P47914	Large ribosomal subunit protein eL29	↓
Q5T6V5	Queuosine 5'-phosphate N-glycosylase/hydrolase	↓

P51151	Ras-related protein Rab-9A	↓

3.2.2 共有差异蛋白功能分析

虽然肥胖组的共有差异蛋白相对于正常组的变化十分显著，且变化趋势统一，但是其中大多数蛋白还未有报道与肥胖、糖尿病等疾病相关。尤其是 Coiled-coil domain-containing protein 194，在 Uniprot 网站上查询不到该蛋白的功能和参与的生物学过程。即便如此，共有差异蛋白中仍有蛋白与肥胖相关，如：Protocadherin beta 具有钙离子结合能力，可能于大脑中特定神经元的建立和维持有关。Protocadherin beta 基因在下丘脑中表达，且能发生具有生物学效应的罕见变异，而在一项涉及 30 位极度肥胖白种成年受试者（平均 BMI = 51.1 kg/m^2）的研究中，通过外周血液白细胞检测发现，此种罕见变异被显著富集，而 BMI 正常人群中未出现此现象[32]，同样该基因的罕见突变在另一项以韩国肥胖儿童为受试者的研究中被显著富集[33]。当通过尿液蛋白质组手段检测时，平均 BMI= 35.79 kg/m^2 的肥胖组即可检测出该蛋白与正常组存在显著差异，侧面说明了尿液蛋白质组的敏感性，以及展现了尿液蛋白质组在寻找早期肥胖标志物方面的潜力。极度肥胖人群还富集到 Olfactory receptor 基因的突变，两基因的变异在极度肥胖中具有协同作用[34]。

这些差异蛋白在肥胖组的变化显著、变化趋势极相同，但是对这些蛋白的研究还都较少，因此，这些差异蛋白值得进一步研究其在肥胖和代谢方面的功能，期以寻找肥胖的生物标志物或潜在药物靶点。

3.2.3 共有差异蛋白功能分析

利用 DAVID 数据库对肥胖组 8-10 个体共有的差异蛋白进行生物学通路的分析。共富集到 15 个 $P < 0.05$ 的生物学过程，结果如图 2 所示。

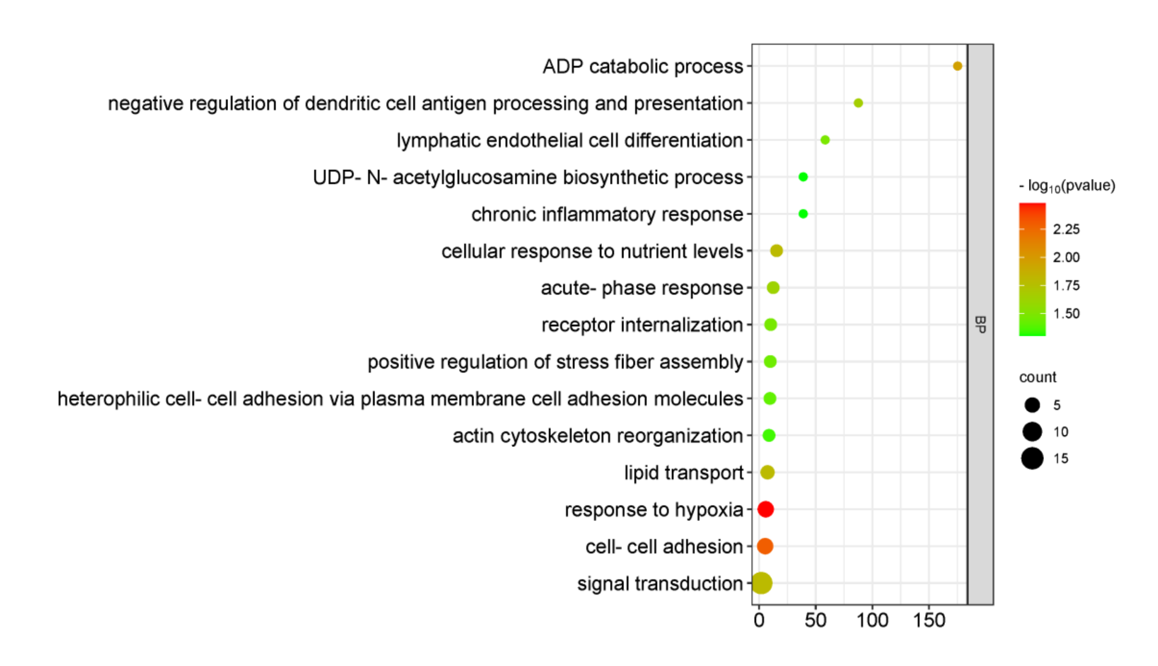


图 2 肥胖组 8-10 个体共有的差异蛋白富集生物学过程

其中包括与营养、代谢相关的生物学过程，如： response to hypoxia，有研究表明内源性缺氧会加重脂肪组织的功能障碍，并刺激炎症分子的分泌，从而导致肥胖[35]、ADP catabolic process 、cellular response to nutrient levels、lipid transport 和 cholesterol metabolic process、UDP-N-acetylglucosamine biosynthetic process，脂肪组织 UDP-N-acetylglucosamine 与 BMI 呈显著正相关，抑制 UDP-N-acetylglucosamine 的生物合成，导致培养的脂肪细胞中葡萄糖刺激的瘦素释放减少[36]。有研究表明与肥胖、糖尿病或癌症相关的葡萄糖代谢失调与该过程中酶水平的增加相关。但是还有一些涉及基因表达和神经系统相关的生物学过程，其 P 值相对于这些生物学过程更小。其中 telomere organization 可能与肥胖相关，有研究表明，端粒长度是生物衰老的强有力标志，并且在肥胖的成年人中注意到端粒磨损增

加[30]。其余生物学过程现在还未查到这些生物学过程与肥胖或 BMI 相关，这为理解肥胖的成因提供了新的研究方向和思路。

3.3 肥胖组个体的个性分析

现有研究已经发现了大量的肥胖生物标志物[1 37 38]。在肥胖组单个个体与正常组尿液蛋白质组差异蛋白中对这些标志物进行检索。很多标志物能够通过尿液蛋白质组差异蛋白反应，且不同个体中出现的标志物并不相同，如表 4 所示。说明尿液蛋白质组具有辅助肥胖的个性化治疗方案制定的潜力。

表 4 肥胖组个体具有的已知肥胖标志物

Biomarker/Sample	Insulin like protein	Insulin like protein Biological process	Lipocalin	Angiotensin-converting enzyme	C-reactive protein
11	Insulin-like growth factor-binding protein 1 Insulin-like growth factor Ⅰ		Lipocalin-1	Angiotensin-converting enzyme	C-reactive protein
12	Insulin growth factor-like family member 1 Insulin-like growth factor-binding protein 5 Insulin-like growth factor-binding protein 1		Lipocalin-1 Neutrophil gelatinase-associated lipocalin	Angiotensin-converting enzyme	C-reactive protein
13	Insulin-like growth factor Ⅱ Insulin-like growth factor-binding protein 4 Insulin-like growth factor-binding protein 2 Insulin-like growth factor-binding protein 3 Insulin-like growth factor-binding protein 1 Insulin-like growth factor-binding protein-like 1	regulation of insulin-like growth factor receptor signaling pathway cellular response to insulin stimulus	Lipocalin-1 Neutrophil gelatinase-associated lipocalin	Angiotensin-converting enzyme	C-reactive protein
14	Insulin-like growth factor-binding protein 2 Insulin-like growth factor-binding protein 3 Insulin-like growth factor-binding protein 4 Insulin-like growth factor-binding protein 1 Insulin-like growth factor Ⅱ Insulin-like growth factor Ⅰ	regulation of insulin-like growth factor receptor signaling pathway	Neutrophil gelatinase-associated lipocalin Lipocalin-1	Angiotensin-converting enzyme	
15	Insulin-like growth factor-binding protein 5 Insulin-like growth factor-binding protein 4 Insulin-like growth factor-binding protein complex acid labile subunit Insulin-like growth factor-binding protein 6 Insulin-like growth factor-binding protein 2 Insulin-like growth factor-binding protein 1 Insulin-like growth factor Ⅱ Insulin-like growth factor Ⅰ Insulin growth factor-like family member 1	regulation of insulin-like growth factor receptor signaling pathway	Neutrophil gelatinase-associated lipocalin	Angiotensin-converting enzyme Angiotensinogen	C-reactive protein
16	Insulin-like growth factor-binding protein 7 Insulin-like growth factor-binding protein 2 Insulin-like growth factor-binding protein complex acid labile subunit Insulin-like growth factor-binding protein 1 Insulin-like growth factor Ⅱ Insulin-like growth factor Ⅰ Insulin growth factor-like family member 1	positive regulation of insulin receptor signaling pathway response to insulin	Neutrophil gelatinase-associated lipocalin	Angiotensinogen	C-reactive protein
17	Insulin-like growth factor-binding protein 4 Insulin-like growth factor-binding protein 5 Insulin growth factor-like family member 1 Insulin-like growth factor-binding protein 1 Insulin-like growth factor Ⅰ	cellular response to insulin stimulus regulation of insulin-like growth factor receptor signaling pathway positive regulation of insulin receptor signaling pathway insulin catabolic process	Lipocalin-1 Neutrophil gelatinase-associated lipocalin	Angiotensinogen Angiotensin-converting enzyme	C-reactive protein
18	Insulin-like growth factor-binding protein 4 Insulin-like growth factor Ⅱ Insulin-like growth factor-binding protein 1 Insulin-like growth factor Ⅰ	cellular response to insulin stimulus	Lipocalin-1 Neutrophil gelatinase-associated lipocalin	Angiotensin-converting enzyme	C-reactive protein
19	Insulin-like growth factor Ⅱ Insulin-like growth factor-binding protein 3 Insulin-like growth factor-binding protein 1			Angiotensinogen	C-reactive protein
20	Insulin-like growth factor-binding protein 5 Insulin-like growth factor Ⅱ Insulin-like growth factor Ⅰ Insulin growth factor-like family member 1		Lipocalin-1		C-reactive protein

Biomarker/Sample	Fatty-acid binding protein	Fatty-acid binding protein Biological process	Coronin 7	Folate	Telomere Biological process
11	Fatty acid-binding protein, intestinal			C-1-tetrahydrofolate synthase, cytoplasmic	
12	Fatty acid-binding protein, intestinal		Coronin-1C		telomere organization
13	Fatty acid synthase		Coronin-1C	Folate receptor beta C-1-tetrahydrofolate synthase, cytoplasmic Cytosolic 10-formyltetrahydrofolate dehydrogenase	telomere organization positive regulation of establishment of protein localization to telomere positive regulation of telomere maintenance via telomerase
14			Coronin-1B Coronin-1C		telomere organization
15	Fatty acid-binding protein, liver Fatty acid-binding protein, intestinal		Coronin-1B Coronin-1C	Folate receptor alpha C-1-tetrahydrofolate synthase, cytoplasmic Cytosolic 10-formyltetrahydrofolate dehydrogenase	telomere organization
16	Fatty acid-binding protein, intestinal Fatty acid synthase Fatty acid-binding protein, adipocyte	fatty acid metabolic process		C-1-tetrahydrofolate synthase, cytoplasmic	telomere organization
17	Fatty acid synthase		Coronin-1C	C-1-tetrahydrofolate synthase, cytoplasmic Cytosolic 10-formyltetrahydrofolate dehydrogenase	telomere organization positive regulation of telomere maintenance via telomerase
18	Fatty acid-binding protein, adipocyte	fatty acid beta-oxidation	Coronin-1C	Folate receptor alpha	
19	Fatty acid-binding protein, adipocyte Fatty acid-binding protein, intestinal	fatty acid beta-oxidation	Coronin-1C	Folate receptor alpha	
20	Fatty acid-binding protein, adipocyte		Coronin-1B Coronin-1C		telomere organization

Biomarker/Sample	Adiponectin	Proprotein Convertase Subtilisin/Kexin Type 1	Angiotensin-converting enzyme Biological process	Resistin	Interleukin-6	Methylation
11	Adiponectin					
12		Proprotein Convertase Subtilisin/Kexin Type 1	angiotensin maturation			
13		Proprotein Convertase Subtilisin/Kexin Type 1	angiotensin maturation		Interleukin-6 receptor subunit beta	
14	Adiponectin					5-methylcytosine rRNA methyltransferase NSUN4
15	Adiponectin	Proprotein Convertase Subtilisin/Kexin Type 1	angiotensin maturation	Resistin	Interleukin-6 receptor subunit beta Interleukin-6 receptor subunit alpha	
16	Adiponectin	Proprotein Convertase Subtilisin/Kexin Type 1				
17			angiotensin maturation	Resistin		
18	Adiponectin			Resistin	Interleukin-6 receptor subunit alpha Interleukin-6 receptor subunit beta	
19	Adiponectin		angiotensin maturation	Resistin		5-methylcytosine rRNA methyltransferase NSUN4
20		Proprotein Convertase Subtilisin/Kexin Type 1		Resistin		

Biomarker/Sample	butyrate	S-adenosylmethionine	S-adenosylmethionine Biological process	Leptin	Wnt/β-catenin pathway
11		S-adenosylmethionine synthase isoform type-2			
12					
13			S-adenosylmethionine cycle		
14	3-hydroxybutyrate dehydrogenase type 2				
15					
16	3-hydroxybutyrate dehydrogenase type 2				
17		S-adenosylmethionine synthase isoform type-2	S-adenosylmethionine cycle		
18				Leptin	
19				Leptin	
20					

Biomarker/Sample	nicotinamide phosphoribosyltransferase	tumor necrosis factor α	Acetylation	Omentin	Uncoupling protein 1	Chemerin	Visfatin	Fat Mass And Obessity Associated gene
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

4 结论

本研究通过 LC-MS/MS 非标记定量的方法，对 10 列肥胖样本与 9 例正常样本进行成组分析和肥胖个人一对多的单人分析筛选差异蛋白，并进行蛋白质功能和生物学过程分析。成组分析筛选差异蛋白和单人分析富集共有差异蛋白，表明尿液蛋白质组能够区分肥胖者和正常者，且肥胖者相对于正常者的部分差异已经被报道与肥胖或代谢相关。单人分析能为肥胖者提供个性化的肥胖线索，辅助提供个性化医疗。通过本研究可以看到尿液蛋白质组在探究肥胖的相关机制、寻找潜在的药物靶点和提供个性化治疗方案等方面的潜力，值得进一步扩大样本量，继续探索。

参考文献

[1] Nimptsch K, Konigorski S, Pischon T. Diagnosis of obesity and use of obesity biomarkers in science and clinical medicine. *Metabolism*. 2019, 92:61-70.

[2] Mayoral LP, Andrade GM, Mayoral EP, Huerta TH, Canseco SP, Rodal Canales FJ, Cabrera-Fuentes HA, Cruz MM, Pérez Santiago AD, Alpuche JJ, Zenteno E, Ruíz HM, Cruz RM, Jeronimo JH, Perez-Campos E. Obesity subtypes, related biomarkers & heterogeneity. *Indian J Med Res*. 2020, 151(1):11-21.

[3] Chen K, Shen Z, Gu W, Lyu Z, Qi X, Mu Y, Ning Y; Meinian Investigator Group. Prevalence of obesity and associated complications in China: A cross-sectional, real-world study in 15.8 million adults. *Diabetes Obes Metab*. 2023, 25(11):3390-3399.

[4] Prillaman M. Why BMI is flawed - and how to redefine obesity. *Nature*. 2023, 622(7982):232-233.

[5] 高友鹤. 尿液有可能成为生物标志物的金矿吗? *中国科学 生命科学*. 2013, 43(8): 708-708

[6] Wei Yin, Weiwei Qin, Youhe Gao Urine glucose levels are disordered before blood glucose level increase was observed in Zucker diabetic fatty rats. *Sci China Life Sci*. 2018, 61:844-8.

[7] Watanabe, Y., et al., Urinary Apolipoprotein C3 Is a Potential Biomarker for Alzheimer's Disease. *Dement Geriatr Cogn Dis Extra*. 2020, 10(3): p. 94-104.

[8] Huan Y, Wei J, Gao Y, et al. Label-Free Liquid Chromatography-Mass Spectrometry Proteomic Analysis of the Urinary Proteome for Measuring the Escitalopram Treatment Response From Major Depressive Disorder. *Front Psychiatry*. 2021, 12:700149.

[9] Meng W, Huan Y, Gao Y. Urinary proteome profiling for children with autism using data-independent acquisition proteomics. *Transl Pediatr*. 2021, 10(7):1765-1778.

[10] Hao Y, Reyes LT, Cheng F, et al. Changes of protein levels in human urine reflect the dysregulation of signaling pathways of chronic kidney disease and its complications. *Sci Rep*. 2020, 10(1):20743.

[11] Ni M, Zhou J, Zhu Z, et al. A Novel Classifier Based on Urinary Proteomics for Distinguishing Between Benign and Malignant Ovarian Tumors. *Front Cell Dev Biol*. 2021, 9:712196.

[12] Travers, S.H., Labarta, J.I., Gargosky, S.E., Rosenfeld, R.G., Jeffers, B.W., Eckel, R.H.. Insulin-like growth factor binding protein-I levels are strongly associated with insulin sensitivity and obesity in early pubertal children. *The Journal of Cinical Endocrinology and Metabolism*. 1998, 83(6):1935e1939.

[13] Wilson, D.. Intraperitoneal fat and insulin resistance in obese adolescents. *Obesity*. 2010, 18(2):402e499.

[14] Chahal, J., Chen, C.-C., Rane, M.J., Moore, J., Barati, M., Song, Y., et al. Regulation of insulin-response element binding protein-1 in obesity and diabetes: potential role in impaired insulin-induced gene transcription. *Endocrinology*. 2008, 149(10):4829e4836.

[15] Brismar, K., Fernqvist-Forbes, E., Wahren, J., Hall, K.. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein- 1 (IGFBP-1), IGFBP-3, and IGF-I in insulin- dependent diabetes. *The Journal of Cinical Endocrinology and Metabolism*. 1994, 79(3):872e878.

[16] Fontana, L., Villareal, D.T., Das, S.K., Smith, S., Meydani, S., Pittas, A., et al. Effects of 2-year calorie restriction on circulating levels of IGF-1, IGFbinding proteins and cortisol in nonobese men and women: a randomized clinical trial. *Aging Cell*. 2016, (15):22e27.

[17] Heald, A.H., Cruickshank, J.K., Riste, L.K., Cade, J., Anderson, S., Greenhalgh, A., et al. Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance

and cardiovascular risk in two populations. *Diabetologia*. 2001, 44(3):333e339.
[18] Gokulakrishnan, K., Velmurugan, K., Ganesan, S., Mohan, V.. Circulating levels of insulin-like growth factor binding protein-1 in relation to insulin resistance, type 2 diabetes mellitus, and metabolic syndrome (Chennai Urban Rural Epidemiology Study 118). *Metabolism*. 2012, 61(1):43e46.
[19] Liew, C.F., Wise, S.D., Yeo, K.P., Lee, K.O.. Insulin-like growth factor binding protein-1 is independently affected by ethnicity, insulin sensitivity, and leptin in healthy, glucose-tolerant young men. *The Journal of Cinical Endocrinology and Metabolism*. 2005, 90(3):1483e1488.
[20] Rajpathak, S.N., McGinn, A.P., Strickler, H.D., Rohan, T.E., Pollak, M., Cappola, A.R., et al. Insulin-like growth factor-(IGF)-axis, inflammation, and glucose intolerance among older adults. *Growth Hormone and IGF Research*. 2008, 18(2):166e173.
[21] Mogul, H.R., Marshall, M., Frey, M., Burke, H.B., Wynn, P.S., Wilker, S., et al. Insulin like growth factor-binding protein-1 as a marker for hyperinsulinemia in obese menopausal women. *The Journal of Cinical Endocrinology and Metabolism*. 1996, 81(12):4492e4495.
[22] Mohamed-Ali, V., Pinkney, J.H., Panahloo, A., Cwyfan-Hughes, S., Holly, J.M., Yudkin, J.S.. Insulin-like growth factor binding protein-1 in NIDDM: relationship with the insulin resistance syndrome. *Clinical Endocrinology (Oxford)*. 1999, 50(2):221e228.
[23] Saitoh, H., Kamoda, T., Nakahara, S., Hirano, T., Nakamura, N.. Serum concentrations of insulin, insulin-like growth factor(IGF)-I, IGF binding protein (IGFBP)-1 and -3 and growth hormone binding protein in obese children: fasting IGFBP-1 is suppressed in normoinsulinaemic obese children. *Clinical Endocrinology (Oxford)*. 1998, 48(4):487e492.
[24] Borai, A., Livingstone, C., Zarif, H., Ferns, G.. Serum insulin-like growth factor binding protein-1: an improvement over other simple indices of insulin sensitivity in the assessment of subjects with normal glucose tolerance. *Annals of Clinical Biochemistry*. 2009, 46(2):109e113.
[25] Sandhu, M.S., Heald, A.H., Gibson, J.M., Cruickshank, J.K., Dunger, D.B., Wareham, N.J.. Mechanisms of disease Circulating concentrations of insulin-like growth factor-I and development of glucose intolerance : a prospective observational study. *Lancet*. 2002, 359:1740e1745.
[26] Lewitt, M.S., Hilding, A., Ostenson, C.-G., Efendic, S., Brismar, K., Hall, K.. Insulin-like growth factor-binding protein-1 in the prediction and development of type 2 diabetes in middle-aged Swedish men. *Diabetologia*. 2008, 51(7):1135e1145.
[27] Petersson, U., Ostgren, C.J., Brudin, L., Brismar, K., Nilsson, P.M.. Low levels of insulin-like growth-factor-binding protein-1 (IGFBP-1) are prospectively associated with the incidence of type 2 diabetes and impaired glucose tolerance (IGT): the Söderåkra Cardiovascular Risk Factor Study. *Diabetes and Metabolism*. 2009, 35(3):198e205.
[28] Lewitt, M.S., Hilding, A., Brismar, K., Efendic, S., Ostenson, C.-G., Hall, K.. IGF-binding protein 1 and abdominal obesity in the development of type 2 diabetes in women. *European Journal of Endocrinology*. 2010, 163(2): 233e242.
[29] Chen Y, Cai GH, Xia B, Wang X, Zhang CC, Xie BC, Shi XC, Liu H, Lu JF, Zhang RX, Zhu MQ, Liu M, Yang SZ, Yang Zhang D, Chu XY, Khan R, Wang YL, Wu JW. Mitochondrial aconitase controls adipogenesis through mediation of cellular ATP production. *FASEB J*. 2020, 34(5):6688-6702.
[30] Gracia CR, Freeman EW, Sammel MD, Lin H, Nelson DB. The relationship between obesity and race on inhibin B during the menopause transition. *Menopause*. 2005, 12(5):559-66.
[31] Raftopoulou C, Paltoglou G, Charmandari E. Association between Telomere Length and Pediatric Obesity: A Systematic Review. *Nutrients*. 2022, 14(6):1244.
[32] Mariman EC, Bouwman FG, Aller EE, van Baak MA, Wang P. High frequency of rare variants with a moderate-to-high predicted biological effect in protocadherin genes of extremely obese. *Genes Nutr*. 2014, 9(3):399.
[33] Moon S, Hwang MY, Jang HB, Han S, Kim YJ, Hwang JY, Lee HJ, Park SI, Song J, Kim BJ. Whole-exome sequencing study reveals common copy number variants in protocadherin genes associated with childhood obesity in Koreans. *Int J Obes (Lond)*. 2017, 41(4):660-663.
[34] Mariman EC, Szklarczyk R, Bouwman FG, Aller EE, van Baak MA, Wang P. Olfactory receptor genes cooperate with protocadherin genes in human extreme obesity. *Genes Nutr*. 2015, 10(4):465.
[35] Wang R, Sun Q, Wu X, Zhang Y, Xing X, Lin K, Feng Y, Wang M, Wang Y, Wang R. Hypoxia as a Double-Edged Sword to Combat Obesity and Comorbidities. *Cells*. 2022, 11(23):3735.
[36] Considine RV, Cooksey RC, Williams LB, Fawcett RL, Zhang P, Ambrosius WT, Whitfield

RM, Jones R, Inman M, Huse J, McClain DA. Hexosamines regulate leptin production in human subcutaneous adipocytes. *J Clin Endocrinol Metab*. 2000, 85(10):3551-6.
[37] Silva-Ochoa AD, Velasteguí E, Falconí IB, García-Solorzano VI, Rendón-Riofrio A, Sanguña-Soliz GA, Vanden Berghe W, Orellana-Manzano A. Metabolic syndrome: Nutri-epigenetic cause or consequence? *Heliyon*. 2023, 9(11):e21106.
[38] Chen N, Wang J. Wnt/β-Catenin Signaling and Obesity. *Front Physiol*. 2018, 9:792.